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PIROPLASMOSIS OF CATTLE IN PANAMA

VALUE OF THE BRAIN FILM IN DIAGNOSIS

HERBERT C. CLARK

From the Board of Health Laboratory, Ancon, Canal Zone

The first important observation relating to piroplasmosis of cattle appears to have been made by Reverly[1] in 1881. He pointed out that the disease was spread by a tick, Boophilus bovis. Babes is credited with having been first to give it microscopic study and to have claimed that it was caused by a parasite (hematococcus bovis) in the red blood corpuscles. He also learned that the disease could be transmitted from sick to healthy animals by means of direct inoculation with blood or kidney substance from an affected animal.

Smith and Kilborne[2] gave the disease its first exhaustive study. The nature, cause, agent and prevention of the disease were so well elucidated by them that but little has been added since save as regards its geographic distribution and the evidence indicating that more than one species of the parasite exist in different parts of the world.

DEMONSTRATION OF PIROPLASMOSIS IN PANAMA CATTLE

"Southern cattle fever" or a disease quite similar to it, has been found in Finland, Norway, Italy, Turkey, Germany, France, Holland, Hungary, England, Portugal, Africa, India, Java, The Philippines, Australia, Mexico, South America (Brazil, Uruguay, Argentina, and Chili), Cuba, and Porto Rico. It would seem reasonable, therefore, to suppose that Panama and other Central American countries should have the disease.

The early construction period of the Panama Canal was not associated with the use of many domestic animals. The meat and dairy products used were imported chiefly from the United States or purchased from the local markets. As the work progressed, draft animals were needed and a comparatively large number of mules and horses were imported. During this period a few small herds of native cattle, hogs, horses, and goats were scattered over the Canal Zone.

Not long after the importation of the mules and horses some of them came in contact with native animals and S. T. Darling,[3] then the director of the Board of Health Laboratory, discovered murrina, a fatal trypanosomal disease of horses and mules. A little later, he also encountered equine piroplasmosis.[4]

Received for publication Oct. 21, 1917.

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[1] Hutyra and Marek: Pathology and Therapeutics of the Diseases of Domestic Animals, 1916, 1, p. 764.

[2] U. S. Dept. of Agriculture, Bureau of Animal Industry, Bull. 1, 1889.

[3] Jour. Infect. Dis., 1911, 8, p. 467.

[4] Ibid., 1913, 13, p. 197.

Near the middle of the construction period, Ancon hospital organized a dairy and stocked it with animals from the United States, chiefly from the gulf coast region. To this dairy were added some imported hogs and large parks of chickens. This enterprise grew to such proportions that it soon required more space and it was removed to a nearby village where it is now known as the Corozal hospital farm. A few cases of tuberculosis were found in the imported cattle, a condition that is almost never found in the native cattle. Acute infectious abortion and other conditions, common elsewhere, were at times encountered among these animals. Hog cholera also made its appearance. It was noted that the calf mortality was higher than is usually expected in well cared for stock. Clinical and post-mortem observation failed to explain this death among the calves and although suspicion was directed at piroplasmosis the presence of the disease could not be established.

The development of the Ancon hospital dairy stimulated local cattle owners in Panama City to more intensive dairy farming and they imported Holstein and Jersey cattle to be crossed with native stock in order to increase the output of dairy products. These animals soon became sick and many of them died. Piroplasmosis was suspected again, but this diagnosis could not be established by microscopic examination. In an outbreak of acute disease in a herd of Holstein cattle, prolonged search was made of the blood films from all the cattle, native and imported, of the dairy in question and Dr. Darling made a necropsy on a Holstein animal. The clinical picture of the disease in the imported animals was that of "southern cattle fever" and in his report (January, 1915) Dr. Darling so indicated, although the only bodies found were some "anaplasma-like" bodies in the blood films of the animal examined after death.

At the close of the construction period of the canal, a supply department was organized and one of the subdivisions of this service is known as the department of cattle industry. Large pastures have been cleared along the course of the canal for the purpose of receiving imported cattle and hogs. Chicken farms were also organized. The imported stock after being fattened in the pasture are slaughtered and furnish the meat supply. Dr. W. J. Taylor of the veterinary staff and Major (now Colonel) F. F. Russel, U. S. Army, the director of the Board of Health laboratory, began to suspect certain cattle in one of these pastures of being ill with "southern cattle fever" although they could not find parasites in the blood films. Nov. 22, 1916, two calves from the Corozal hospital farm in a moribund condition were killed and examined with the following results:

CALF 1220.—Emaciated, yellow heifer, about 10 months old, weight 200 lbs. The hide was tick infested. There was extreme degree of anemia but no sign of jaundice or of hemoglobinuria. An acute fibrino-purulent peritonitis was present in the upper half of the cavity and in the upper end of the small bowel several large patches of a fibrinous exudate were found. No marked change in the spleen. Parenchymatous degeneration in the liver, kidneys, and heart muscle, but no petechial hemorrhages in any of the serous membranes. Films were prepared from the various organs and the blood, and stained with Hastings' modification of the Romanowsky method. A scant number of Babesia bigemina were found chiefly in the bone marrow of the rib and the crushed gray matter of the brain cortex.

The intestinal and peritoneal lesions may have had much to do with the death of this animal and the piroplasmosis may have been only of the chronic type.

CALF 1221.—A small heifer, weighing 35 lbs., about 6 months old. The hide was tick infested; the animal was extremely emaciated, very anemic, but there was no jaundice or hemoglobinuria. All serous membranes contained areas of ecchymosis, and the acute parenchymatous degeneration was extreme. The spleen was enlarged, the capsule tense, the pulp soft, oozing, and purple. The brain did not show any sign of lesion except an intense degree of congestion.

Films from the various organs in this case all revealed Babesia bigemina, but the films showing the greatest abundance were those from the gray matter of the brain cortex and ganglionic matter at the base. These preparations showed capillaries literally stuffed with paired, pyriform bodies. Some of these films were sent to Dr. Theobald Smith who confirmed the belief that the parasites were Babesia bigemina.

Four weak, emaciated, tick infested calves soon arrived from the Miraflores pasture of the supply department and these were kept under observation from a few days to 3 weeks. They had fever but did not show the typical curve of the acute disease. Blood films were examined each morning and evening and in one case revealed a few parasites in the peripheral blood as well as in preparations made from a blood engorged female tick. An effort to further utilize this method of examination did not prove practical. No jaundice or "red water" was noted in these calves while under observation, but they may have had periods of hemoglobinuria since it was not possible to observe all voidings. The reduction in the hemoglobinuria was marked since the average reading was 50%. The ultimate diagnosis of piroplasmosis was made at necropsy. All very sick animals in this particular pasture were now located and kept out of the herd. A tick eradication was then instituted along the lines employed in the gulf coast region of the United States. Ticks from some of the animals were studied by L. H. Dunn, entomologist, who reported them to be Margaropus annulatus australis.

In the meantime I examined 25 more animals that had been taken away from the herd, all calves from 6-10 months of age except 2 old native cows. In all 29 cattle were examined and the film study except in 4 cases included only those prepared from the brain, spleen, marrow, and blood.

The following results were obtained as to the presence of Babesia bigemina:

	Positive	Negative
Brain films........................	29	0
Marrow films, rib.................	10	19
Spleen films.......................	8	21
Blood films........................	3	26

For some reason the parasites were easier to find or were more numerous in the brain capillaries than in the other 3 preparations. The constant presence of the parasites in the brain capillaries in this disease is even more striking than in the case of the parasite of malaria in the human being.

The cattle thus far in question were born on the Isthmus, and most of them were the calves of native cows bred to an imported bull. Since birth they had been exposed to tick infestation. The universal presence of the parasite in these animals indicated that a latent type of the disease might be common among the native cattle outside the Canal Zone.

Through the kindness of Dr. Baltasar Bosch, city veterinarian in Panama, and of Dr. W. J. Taylor, veterinarian in charge of the cattle industry in the Canal Zone, I was permitted to study an unselected series of prime beef animals immediately after they were killed. Further experience with films of various tissue, including the heart, kidney, and liver, led me to continue the use of brain films.

Three or four blows with an ax, hatchet, or machete just behind the horns will expose the brain, and as a rule the dura and the brain are not badly lacerated. A piece of the gray matter of the brain cortex about the size of a split pea was selected, placed on a glass microscope slide near its middle, another slide being used to crush it so that a film extends over half the slide. By increasing the pressure as one drags the material toward the ends of the slide it is possible to leave a film that is thick at the middle of the slide and thin at the end. At some place in the preparation networks of blood-filled capillaries will be found. The film is quickly dried and then stained as soon as possible in some polychrome blood stain. When ready for examination use low magnification to find some well defined thin areas containing capillaries, and then a high magnification. The capillaries can then be traced for blood cells containing parasites. Many times the first field of capillaries examined in the slaughter-house revealed one or more parasites. The average time spent in searching in a particular case was 3 minutes, although some of the cases required 20-30 minutes.

The beef cattle of Panama came from all parts of the cattle region of the Republic. They were examined in lots of 20-25 just in the order that the authorities chose to kill them without regard to age or degree of tick infestation. Every animal of the 125 examined showed Babesia bigemina and in spite of a rather fair degree of infection in some offered no clinical sign of the disease.

The supply department imports a similar class of animals from Colombia which are slaughtered at Cristobal. An examination of 150 of these showed that 90% were carriers of the same parasite.

Other opportunities have been taken to examine cattle of all types in outlying regions as well as in the basin of the Chagres river as far up the river as inhabitants are to be found. In all localities the disease was present. Native cattle suffering serious injury, long periods of starvation, etc., develop piroplasmosis, at least their examination showed an abundance of parasites.

It appears from these results that almost all the cattle of Panama and Colombia must be considered as carriers of the parasites of piroplasmosis. Since no protective measures are employed against the disease and since the tick in this locality continues an almost uninterrupted cycle of development throughout the year, all cattle born here are exposed to the infection from the time of their birth on. It is well known that calves survive the disease better than the fullgrown cattle that have not had the disease in their early life, nevertheless, it is possible that this disease partially explains the high death rate in calves in this country. The animals that do survive the calf period certainly acquire an immunity that protects them from future attacks provided injury, starvation, or some intercurrent disease does not undermine the vitality. The importation of nonimmune cattle, when not followed by protective measures, is surely followed by considerable loss. Generally speaking, the native cattle (although hardy animals) do not fill out into the fat sleek animals commonly found outside a tick belt.

PECULIARITIES OF PIROPLASMOSIS OF PANAMA CATTLE

The acute cases found in Panama, as a rule, occur in nomimmune imported cattle. Many of the fullgrown animals of this class are lost, but a fair percentage of the calves survive. The clinical picture of the acute disease is like that described by Smith and Kilborne.[2] Native animals, as already mentioned, suffer acute attacks when they are subjected to starvation, injury, or an association of diseases. In the chronic form the animal appears wasted, anemic, and lazy, with a tick-infested hide. Jaundice and hemoglobinuria are not often noted, and the temperature curve is not characteristic. Carriers of the parasite may not show a single sign of the disease except possibly the fact that they do not become large fat animals.

In acute cases there is often a lemon colored conjunctiva and oral mucous membrane. Punctate hemorrhages in these regions are not infrequent. The number of ticks present may not seem noteworthy. Cattle dying of the more chronic type are often heavily infested with ticks and may show extreme wasting and anemia.

The head and neck often offer little that helps to differentiate the disease. There is no sign of acute inflammation. Acute cases reveal intense congestion of the meninges and gray matter often with miliary and larger hemorrhages. In chronic cases the brain is usually pale, with increase in fluid. The most important feature is finding the parasites in the brain capillaries, in acute as well as chronic types of the disease. My experience does not indicate the meninges and choroid plexus to be as satisfactory as the brain film already mentioned.

It appears from the report of Smith and Kilborne[2] that the brain was examined in only 4 of their cases, and here they found great numbers of parasites in the pia, choroid plexus and the brain substance. Since these were probably acute cases, this organ did not impress them as a suitable one for routine examination.

Punctate hemorrhages in the lungs may be found in acute cases and frequently areas of pneumonia are present and are covered on the pleural surface by a granular exudate. Chronic cases usually show pale somewhat edematous lungs. In acute cases there may be extensive areas of ecchymosis in the myocardium, pericardium, and endocardium. At times there is a yellow tint in the endocardium. Chronic cases as a rule show an increase of fluid in the pericardium, and a pale soft heart muscle. Almost all animals have sarcosporidia, the sporozoits being found in large numbers in films from the heart muscle. The abdominal wall is usually edematous, with smaller and larger hemorrhages; minute hemorrhages occur frequently in the peritoneal coat of the stomach and intestines. In chronic cases there may be some effusion in the peritoneal cavity.

In acute cases there is an enormous enlargement of the spleen with a tight, smooth capsule, sprinkled with punctate hemorrhages. The color of the capsule is darker than normal. The pulp is purple, semidiffluent, and oozes so much that the malpighian bodies are hidden. It is difficult to say anything definite about the spleen in chronic cases because all native cattle harbor several types of latent infections that may play some rôle in modifying the spleen. The organ usually is a little enlarged, the consistency increased, the capsule is thicker than normal and almost white in color, wrinkling easily.

In acute cases there is marked enlargement of the liver, which has a mottled brown and yellow color, the margin rounded, pressure necrosis being present at times. The bile is usually thick, the gallbladder frequently distended. Chronic cases present a pale liver without noticeable increase in size.

In acute cases the kidney is greatly swollen, mahogany colored, sometimes sprinkled with small hemorrhages, the cut surface of the cortex showing brown and yellow striations. Hemoglobinuria is occasionally present; albumin is present in large amounts. Chronic cases have a pale yellow kidney, sometimes enlarged; there may or may not be albuminuria, and hemoglobinuria is seldom seen.

Intestinal parasites and inflammatory processes are so common among cattle that it is difficult to determine the influence of this disease on the variety of lesions found. In calves only congestion and minute hemorrhages in the serous coat have been found and it is probable that nothing more than this is caused by the disease in older cattle.

There is a marked reduction in the hemoglobin in both the acute and chronic forms of the disease. In other parts of the world from 1-10% of the red corpuscles in the peripheral blood are said to be infected. The puzzling feature of the disease in Panama is that in never in more than 8 or 10 cases have I been able to find the parasite in the peripheral blood, even in acute cases. I attribute our delayed identification of the disease in this region to the extreme scarcity of the parasite in the peripheral blood and to our failure to open the cranial cavity in animals that died of the disease. Concerning the distribution of the parasites in the body Smith and Kilbourne[2] report as follows: "They are very abundant as determined thus far, in the capillary blood of the heart muscle, but quite rare in the skeletal muscles. Of the internal organs the kidneys usually contain the largest numbers, not infrequently from 50-80% of all corpuscles are infected. Next come the liver and spleen. In spite of the fact that the spleen is loaded with red corpuscles by several times its own weight, rarely more than one-tenth contain parasites. Infected corpuscles have been found in great abundance in the capillaries of the choroid plexus and in the vessels of the pia and the brain substance. They have also been found in the capillaries of the intestinal mucosa.

They further report that: "The brain was removed in a small number of cases and carefully examined, but no lesions which can be regarded as peculiar to or characteristic of the disease were observed. It may be said, in general, that the brain shared the general tendency toward the injection of the capillary system. The gray matter of the cerebrum and especially of the cerebellum appeared of a more pinkish color." It would appear that the brain was not given that routine film study that was accorded the heart muscle, kidney, liver, etc.

Local experience with acute cases in imported stock (initial attack) largely confirm the statements just quoted, but notwithstanding the fact that an abundance of parasites can be found in films prepared from the heart and kidney, the brain has always revealed a greater abundance, and when chronic cases and carriers are to be examined at necropsy or in the abattoirs the system of brain capillaries take precedence over all others. It is reasonable to suppose that organs with intricate capillary systems favor the presence of such parasites, but the capillaries in the kidney, heart muscle, liver, spleen, etc., do not lend themselves readily to the preparation of films free of débris and which offer a sufficient number of intact small capillaries. The brain cortex is rich in small capillaries, and by selecting a bit about half the size of a pea, one can press it between 2 glass slides and drag them apart with increasing pressure as the ends are approached so that a thinner and thinner film is made. Now somewhere in such film, usually about the middle, an abundant network of capillaries is found. The slide should be dried quickly and stained at once if possible. Any modification of the polychrome used as customary for staining blood will answer. Russell's[5] method is highly recommended if a large number are to be examined, being a good field method since it saves time and stain, and wide-mouthed bottles serve for staining jars. In a brain film practically all the blood cells will be held inside the capillaries which thus isolate the blood from confusing elements as in other tissue films. The capillary wall does not prevent the penetration of the stain. The number and small size of the cerebral capillaries form a good seine in which infected blood cells are caught. Acute cases will show all capillaries in the brain film full of infected red cells. Chronic cases and especially carriers may require the tracing of several capillary networks. In chronic cases and carriers, such as steers, I have scores of times readily found the parasites in the brain film when the films from all other organs were negative after a long search.

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[5] Jour. Am. Med. Assn., 1915, 64, p. 2131.

The common type found in the brain is the pair of pyriform bodies lying so that their sharp ends approximate or even appear to touch. The two bodies usually form a rather acute angle, but the degree of this angle may vary greatly, in fact, many times they appear almost in a straight line. The two bodies may vary in both size and shape to some extent. Some cases show a modification of the pyriform shape that closely approaches a diamond or kite shape. In such cases, however, the large end of the body has a much more blunt point than the small end. The polychrome stain gives the parasite a blue color and the center of the large end is lighter in color than the rest. A well circumscribed red dot is frequently found in the large end of the paired bodies and it is somewhat laterally located. Frequently this red material is not collected into a discrete body, but may appear as granules arranged in a diffuse manner chiefly in the large end of the parasite.

Films that have been dried slowly before staining or that have been prepared several hours post mortem will show the parasites staining a solid blue color and contracted in appearance. Short blunt pyriform pairs and even larger diplococcal bodies are common.

A greater variation in the size and morphology of the parasite has been found in films from the marrow of the rib. Here also the common type is the paired pyriform body, but the variation in the size of the 2 bodies, in the angles formed by them, and even in the relation of the 2 bodies to each other in the cell is quite remarkable. Sometimes they are parallel, sometimes in reverse directions, and at other times they occupy totally different parts of the cell.

Single pyriform types and comparatively large irregular round forms that simulate the parasite of malaria are infrequently seen.

The type in the peripheral blood film of a few acute cases were single pyriform bodies larger than one of the paired forms found in the brain.

So few "anaplasma-like" bodies were found in the blood films and their position was so inconstant that it is unsafe to say that the bodies really represent parasites.

The average size of the typical paired pyriform bodies found in an acute case in the brain measured 3.6 by 1.8μ.

The following animals were inoculated with the defibrinated blood from beef cattle known to be *carriers* of Babesia bigemina: 4 guinea-pigs, 1 chicken, 2 pigeons, 1 monkey, 3 opossums, 2 young dogs, 1 mule, 1 goat, and 2 nonimmune calves. The calves developed the disease, but no results were obtained in any of the other animals.

One faces many difficulties in the study of a particular disease in native cattle because these animals are the hosts to so many parasites. The skin, muscles, blood, respiratory tract and alimentary tract all have their parasites. In making observations on piroplasmosis it has been found that sarcosporidiosis, filariasis, trypanosomiasis were all about as prevalent as piroplasmosis. Dr. Oscar Teague and the author have succeeded in almost every attempt to grow a large trypanosome from the slaughter-house animals. I have found a spirochete in the blood film of one animal and Dr. Teague has grown one from another animal. They are probably S. theileri.

There is a striking absence of tuberculosis and of the cysticercus in the native cattle.

Anthrax was noted by Darling and Bates in the early years, but the first real epidemic occurred recently and was placed under control by the efforts of Major (now Col.) Russel and Dr. W. J. Taylor. Actinomycosis has been seen once in native cattle.

While visiting some outlying districts and the basin of the Chagres River I had the opportunity to examine wild animals, among them 10 deer; 4 of the deer contained parasites that seemed to be identical with the piroplasma in cattle.

About 24 imported hunting dogs have been examined and 3 of them have shown Piroplasma canis.

Panama is a tropical country and therefore favors tick development without much seasonal interruption. Native cattle run at large and are at no time stable fed or protected unless used for dairy purposes or as draft animals. Preventive measures now in use at the Corozal hospital and farm and the Miraflores pasture of the supply department show that piroplasmosis and tick infestation can be controlled to a large extent even in Panama. Although native animals are fairly secure in their acquired immunity, it will be interesting to see what results will be obtained when tick eradication is undertaken. Certainly it seems reasonable to expect a drop in calf mortality, an increase in the weight of beef animals, and a greater output in dairy products. Prevention and protective measures have proved their value in other places.

SUMMARY

Piroplasmosis of cattle is present and practically speaking universal in Panama.

Nonimmune cattle when imported into Panama contract the disease and many of them die.

A positive antemortem diagnosis of piroplasmosis in this locality is extremely difficult on account of the scarcity of the parasites in the peripheral blood.

The examination of the blood in films of the gray matter of the brain makes the detection of the parasite at necropsy and in the abattoir comparatively easy.

In Panama piroplasmosis has been found in the horse, cattle, deer, and dog.

Investigations of native cattle led to the observation that they are hosts in almost every instance to Babesia bigemina, filaria, sarcosporidia, and a large trypanosome, probably of a harmless type. Two cattle revealed a spirochete, probably S. theileri.